

mAb competition assay

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 An abbreviated version of this protocol was published in Science Translational Medicine in Mar 2022

Human antibody recognizing a quaternary epitope in the Puumala virus glycoprotein provides broad protection against orthohantaviruses

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Detailed protocol

mAb competition assay

1. Incubate 100 μ l of 100 nM test IgG with 2 μ l of goat anti-human IgG capture beads (Spherotech Goat anti-Human IgG beads, catalog # HUP-60-5) for 20 minutes at room temperature. Prepare in a 96-well v-bottom plate.
2. Centrifuge beads at 2400 \times g for 3 min to pellet and remove supernatant.
3. Wash beads with 200 μ l of PBS+0.1% BSA, centrifuge beads and remove supernatant.
4. Pre-incubate 50 nM biotinylated Gn H/Gc with 1 μ M competitor F(ab) for 30 minutes at room temperature in a total volume of 50 μ l and then combine with the IgG-loaded beads.
5. Incubate for 5 minutes at room temperature.
6. Wash beads by adding 200 μ l of PBS+0.1% BSA, centrifuge, and remove supernatant.
7. Repeat wash.
8. Resuspend beads in 50 μ l of streptavidin-Alexa Fluor 633 (Life Technologies) diluted 1:500 in PBS+0.1% BSA. Incubate for 20 minutes on ice. IgG loading on the bead can be detected by including anti-LC-FITC (Southern Biotech catalog #2062-02) diluted 1:100 into the staining mix.
9. Wash beads with 200 μ l of PBS+0.1% BSA, centrifuge beads and remove supernatant.
10. Repeat wash.
11. Resuspend beads in 100 μ l of PBS+0.1% BSA and analyze by flow cytometry using a FACSCanto II (BD Biosciences) or comparable instrument.
12. Assess competition by measuring the fold reduction in PUUV Gn H/Gc binding in the presence of competitor F(ab) relative to an antigen-only control. Antibodies were considered competitors when a greater than five-fold reduction was observed in the presence of control F(ab) relative to an antigen-only control.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Strandin, T. , Herbert, A. , Forsell, M. , Walker, L. and Chandran, K. (2022). mAb competition assay. Bio-protocol Preprint. bio-protocol.org/prep1630.
2. Mittler, E., Wec, A. Z., Tynell, J., Guardado-Calvo, P., Wigren-Byström, J., Polanco, L. C., O'Brien, C. M., Slough, M. M., Abelson, D. M., Serris, A., Sakharkar, M., Pehau-Arnaudet, G., Bakken, R. R., Geoghegan, J. C., Jangra, R. K., Keller, M., Zeitlin, L., Vapalahti, O., Ulrich, R. G., Bornholdt, Z. A., Ahlm, C., Rey, F. A., Dye, J. M., Bradfute, S. B., Strandin, T., Herbert, A. S., Forsell, M. N. E., Walker, L. M. and Chandran, K. (2022). Human antibody recognizing a quaternary epitope in the Puumala virus glycoprotein provides broad protection against orthohantaviruses. Science Translational Medicine 14(636). DOI: [10.1126/scitranslmed.abl5399](https://doi.org/10.1126/scitranslmed.abl5399)

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